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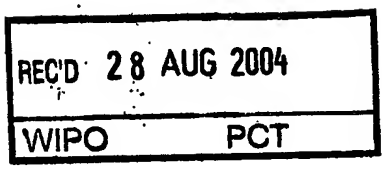

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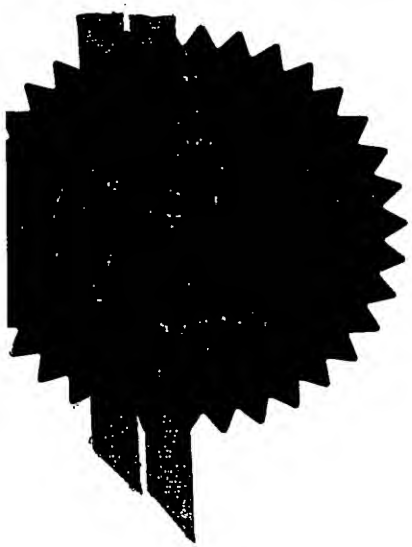

Signed P. Mahoney

Dated 7 May 2004

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Patents Form 1/77

Patents Act 1977
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21JUL03 E823795-1 000192
P01/7700 0.00-0316910.9

Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference — N.89333 JHS

2. Patent application number *(The Patent Office will fill in this part)* — 0316910.9 — 18 JUL 2003

3. Full name, address and postcode of the or of each applicant *(underline all surnames)*

SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE SPA
47, Viale Shakespeare
00144 Rome, ITALY

Patents ADP number *(if you know it)* 7209610001

If the applicant is a corporate body, give the country/state of its incorporation — IT

4. Title of the invention — FLUOROCOMBRETASTATIN AND DERIVATIVES THEREOF

5. Name of your agent *(if you have one)* — J.A. KEMP & CO.

"Address for service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

14 South Square
Gray's Inn
London
WC1R 5JJ

Patents ADP number *(if you know it)* 26001

6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and *(if you know it)* the or each application number

Country	Priority application number *(if you know it)*	Date of filing *(day / month / year)*

7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application

Number of earlier application	Date of filing *(day / month / year)*

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'Yes' if:*

a) any applicant named in part 3 is not an inventor, or
b) there is an inventor who is not named as an applicant, or
c) any named applicant is a corporate body.
See note (d))

YES

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form. Do not count copies of the same document

Continuation sheets of this form	
Description	23
Claim(s)	4
Abstract	1
Drawing(s)	5 + S

10. If you are also filing any of the following, state how many against each item.

Priority documents	
Translations of priority documents	
Statement of inventorship and right to grant of a patent *(Patents Form 7/77)*	7
Request for preliminary examination and search *(Patents Form 9/77)*	1
Request for substantive examination *(Patents Form 10/77)*	
Any other documents *(please specify)*	Covering letter

11. I/We request the grant of a patent on the basis of this application.

Signature J.A. KEMP & CO. Date 18 July 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

SEXTON, Jane Helen
020 7405 3292

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Patents Form 7/77

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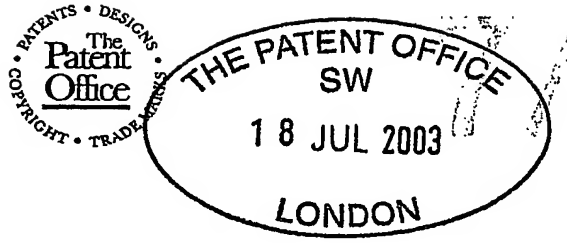

Statement of inventorship and of right to grant of a patent

The Patent Office

Cardiff Road
Newport
South Wales
NP10 8QQ

1. Your reference	N.89333 JHS	
2. Patent application number *(if you know it)*	0316910.9	18 JUL 2003
3. Full name of the or of each applicant	SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE SPA	
4. Title of the invention	FLUOROCOMBRETASTATIN AND DERIVATIVES THEREOF	
5. State how the applicant *(s)* derived the right from the inventor *(s)* to be granted a patent	By assigment	
6. How many, if any, additional Patents Forms 7/77 are attached to this form? *(see note (c))*	6	
7.	I/We believe that the person *(s)* named over the page *(and on any extra copies of this form)* is/are the inventor *(s)* of the invention which the above patent application relates to. Signature J.A. KEMP & CO.	Date 18 July 2003
8. Name and daytime telephone number of person to contact in the United Kingdom	SEXTON, Jane Helen 020 7405 3292	

Notes

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Enter the full names, addresses and postcodes of the inventors in the boxes and underline the surnames

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Patents ADP number *(if you know it)*: 8676595001

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Patents ADP number *(if you know it)*: 8676637001

Reminder

Have you signed the form?

Fluorocombretastatin and derivatives thereof

Background of the invention

Neoplastic diseases, characterized by the proliferation of cells which are not subject to normal cell proliferating controls, are a major cause of death in humans and other mammals. Cancer chemotherapy has provided new and more effective drugs to treat these diseases and has also demonstrated that drugs which disrupt microtubule synthesis are effective in inhibiting the proliferation of neoplastic cells.

Microtubules play a key role in the regulation of cell architecture, metabolism and division. The microtubule systems of eukaryotic cells comprises a dynamic assembly and disassembly matrix in which heterodimers of tubulin polymerize to form microtubules in both normal and neoplastic cells. Within neoplastic cells, tubulin is polymerized into microtubules which form the mitotic spindle. The microtubules are then depolimerized when the mitotic spindle's use has been fulfilled. Agents which disrupt the polymerization or depolymerization of microtubules in neoplastic cells, thereby inhibiting the proliferation of these cells, comprise some of the most effective cancer chemotherapeutic agents in use.

Combretastatin A-4 (CA-4), isolated from the African bush willow, Combretum caffrum (Combretaceae) (*Pettit, G.R., et al.; Experientia, 1989, 45, 209*) shows exciting potential as an anticancer agent binding strongly to tubulin at a site shared with, or close to, the colchicine binding site (*Lin, C.N., et al; Biochemistry, 1989, 28, 6984*). The bond to tubulin prevents its polymerization into microtubules with anti-mitotic effect. CA-4 inhibits cell growth at as low as nanomolar concentrations and shares many structural features common to other tubulin-binding agents such as colchicine and podophyllotoxin.

The phosphate salt [CA-4P] (*Pettit, G.R., et al.; Anticancer Drug Des., 1995, 10, 299*), which has better water solubility than CA-4, has

entered Phase II clinical trials.

It is the ability of combretastatins to damage tumor vasculature, thereby effectively starving tumors of nutrients, which makes them such exciting molecules.

Recently many studies have shown that a number of antiangiogenic agents, like CA-4P, can inhibit retinal neovascularization in a well-characterized murine model of ischemia-induced proliferative retinopathy.

These studies suggest that as CA-4P or new derivatives as other antiangiogenic agents, could be useful in the treatment of non-neoplastic diseases like ischemia-induced proliferative retinopathy (*Griggs, J., et al., Am. J. Pathol., 2002, 160(3), 1097-103*).

The spatial relationship between the two aromatic rings of combretastatin, colchicine and similar drugs is an important structural feature that determines their ability to bind to tubulin (*McGown, A.T., et al., a) Bioorg. Med. Chem. Lett., 1988, 8(9), 1051-6; b) Bioorg. Med. Chem. Lett., 2001, 11(1), 51-4*).

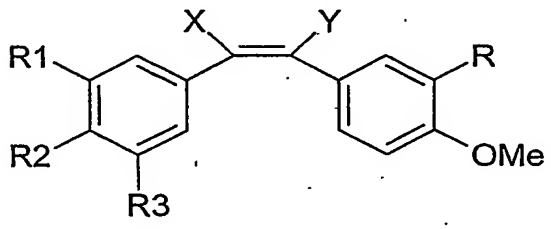
Since '80s researchers have discovered that the selective introduction of fluorine into biologically active molecules exerts an influence on activity. Therefore, important endeavour in drug design has been described and a number of compounds incorporating fluorine as a bioisosteric replacement for hydrogen were reported (*Giannini, G., Current Medicinal Chemistry, 2002, 9, 687-712*).

Summary of the invention

It has now been found that without any modification of the cis-stilbene motif the introduction of the strongly electron-withdrawing fluorine atom in olefin bond allows the biological activity to increase or, in case of the same activity, to influence the pharmacodynamics activity.

Monofluorinated and 1,2-difluoro stilbenes have been synthesized.

Accordingly, it is an object of the present invention a compound of formula (I)



wherein:

R_1, R_2 and R_3, which can be the same or different, are H, OMe, NO_2, NHR';

X and Y are halogen or H, but cannot be both H;

R = OH, OPO_3Na_2, NO_2, NHR';

R' = H, alkyl (C_1-C_6), $(COCHR''NH)_n$-H;

R'' = H, an amino acid side chain, Ph;

n an integer comprised between 1 and 3;

their pharmaceutically acceptable salts, racemates and single enantiomers.

Other objects of the present invention are processes for the preparation of the compounds of the above Formula (I).

Another object of the present invention is the use of the compounds of Formula (I) as test compounds in a biological assay for microtubule

polymerization.

The compounds of Formula (I) have antitubulin activity at least comparable to that of CA-4 (*J. Med. Chem*, **2002**, 45:1697-1711).

Another object of the present invention is the use of the compounds of Formula (I) as medicaments, in particular for the preparation of a medicament for treating pathological states which arise from or are exacerbated by cell proliferation.

A further object of the present invention are pharmaceutical compositions comprising at least a compound of Formula (I) as active ingredient in admixture with at least one pharmaceutically acceptable carrier and/or excipient.

These and other objects of the present invention shall be illustrated in detail also my means of Examples and Drawings, wherein, in the latter:

Figure 1: synthesis of difluorocombretastatin;
Figure 2: synthesis of difluoronitro- and difluoroaminocombretastatin;
Figure 3 : synthesis of monofluorocombretastatin;
Figure 4: synthesis of difluorocombretastatin disodium-phosphate;
Figure 5: synthesis of bromofluorocombretastatin.

It shall be understood by the skilled person that in the Figures 1-5 synthetic schemes are provided for the preferred compounds of the present invention, but the skilled reader will understand that these schemes are applicable to the whole range of the invention, just selecting the appropriate starting materials, depending on the meanings in Formula (I), and resorting to the general common knowledge for the obvious modifications of the reaction conditions and reactants.

Detailed description of the invention

According to the present invention, R" is preferably the side chain of a natural amino acid, and in particular Ala, Asn, Asp, Cys, Gly, Gln,Glu, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Tyr, Try, Val.

Particularly preferred compounds are those of formula (I) wherein:
at least one of X and Y is halogen, R_1-R_3 are methoxy, and R is hydroxy;
at least one of X and Y is halogen, R_1-R_3 are methoxy, R is amino or substituted amino;
at least one of X and Y is halogen, R_1-R_3 are different from methoxy, R is hydroxy;
R is OPO_3Na_2;
R' is $(COCHR"NH)_n$-H.

Particularly preferred compounds are those wherein:
X = Y = F; R = OPO_3Na_2: difluorocombretastatin;
X = Y = F; R = NH_2: difluoroaminocombretastatin;
X = H; Y = F; R = OPO_3Na_2: monofluorocombretastatin;
X = F; Y = H; R = OPO_3Na_2: monofluorocombretastatin;
X, Y = Br, F; R = OH: bromofluorocombretastatin;
X = H; Y = F; R = NH_2: monofluoroaminocombretastatin;
X = F; Y = H; R = NH_2: monofluoroaminocombretastatin.

Processes for the preparation of the compounds of the present invention shall be described in details, by making reference to the synthetic schemes appended as Figures, wherein:

The compounds of the present invention can be prepared by conventional synthetic methods, however, in some preferred embodiments of the present invention, the starting compound is a compound of formula (I), wherein both X and Y are hydrogen.

A process for the preparation of compounds of Formula (I), wherein X and Y are both F comprises the following steps:
a) reaction of 1-bromo-1,2-difluoro-2-(4-methoxy-3-(protected OH)-phenyl)ethene with 3-R_1-4-R_2-5-R_3-phenylboronic acid, and

b) restoring the 3-(protected OH) group.

For step a), 1-bromo-1,2-difluoro-2-(4-methoxy-3-(protected OH)-phenyl)ethene can be obtained by synthetic methods available in the art. For example, isovanillin, with OH group suitably protected, is transformed into 1-bromo-1,2-difluoro-2-(4-methoxy-3-(protected OH)-phenyl)ethene.

Isovanillin is a commercially available product, as well as the 3,4,5-trisubstituted-phenyl-boronic acid is commercially available, or can be obtained by conventional methods. Also many other mono-, di-, and tri-substituted -phenyl-boronic acids are commercially available. However, the starting materials can be obtained by conventional methods.

Reaction of step a) is carried out in a suitable reaction medium, for example an organic solvent, or a mixture of water and the solvent, in the presence of aqueous base, for example an alkaline carbonate. The use of a catalyst can be advisable, and a preferred example is $Pd(Ph_3P)_4$. The reaction temperature is selected according to the starting materials, the solvent and the catalyst used. Preferably, the reaction temperature is at the reflux temperature of the reaction medium.

Removal of the protecting moiety from the hydroxy group is absolutely conventional and is normally performed by the person skilled in the art. A preferred protecting group is found among commercially available organosilyloxy derivatives, for example tert-butyl-dimethyl-syliloxyphenyl. Removal of such groups is done with conventional methods.

A process for the preparation of compounds of Formula (I), wherein one of the X and Y is F and the other one is hydrogen, comprises the following steps:

a) bromofluorination of the compound of Formula (I), wherein X and Y are H, and

b) base-promoted HBr elimination.

This process is disclosed in *Giannini, G., Gazz. Chim. It., 1997, 127, 545; Thakker D.R., et al., J. Org. Chem., 1989, 54, 3091.*

Compounds of Formula (I), wherein one of the X and Y is F can be also prepared by a process comprising the following steps:
a) transformation of compound of Formula (I), wherein X and Y are H into the respective bromohydrin, and
b) base-promoted HBr elimination.

This process is disclosed in *Giannini, G., Gazz. Chim. It., 1997, 127, 545; Thakker D.R., et al., J. Org. Chem., 1989, 54, 3091.*

In alternative, compounds of Formula (I), wherein one of the X and Y is F can be prepared by a process comprising the following steps:
a) transformation of compound of Formula (I), wherein X and Y are H into the respective epoxide;
b) epoxide opening to give the respective bromohydrin, and
c) base-promoted HBr elimination, or in alternative,
d) epoxide opening to give the respective fluorohydrin, and
e) elimination of the opportune hydroxyl derivative.

This process is disclosed in *Giannini, G., Gazz. Chim. It., 1997, 127, 545; Thakker D.R., et al., J. Org. Chem., 1989, 54, 3091.*

Compounds of Formula (I), wherein one of the X or Y is F and the other is Br are prepared by a process comprising the following steps:
a) transformation of compound of Formula (I), wherein X and Y are H into the respective bromohydrin, and
b) base-promoted HBr elimination.

This process is disclosed in *Giannini, G., Gazz. Chim. It., 1997, 127, 545; Thakker D.R., et al., J. Org. Chem., 1989, 54, 3091.*

In a preferred embodiment, the starting compound is Combretastatin A (Formula I, R_1, R_2, R_3 = OMe, X and Y = H, R = OH).

Pharmaceutically acceptable salts are obtained with conventional methods reported in the literature and do not require any further description.

As above disclosed, the compounds of the present invention are useful as medicaments, and, due to their activity on tubulin site, they can be used for the preparation of a medicament for the treatment of pathological states which arise from or are exacerbated by cell proliferation.

An example of said pathological state is a tumour, and among them, both solid and haematic tumors can be treated, for example sarcoma, carcinoma, carcinoid, bone tumour, neuroendocrine tumour, lymphoid leukaemia, acute promyelocytic leukaemia, myeloid leukaemia, monocytic leukaemia, megakaryoblastic leukaemia and Hodgkin's disease.

In another aspect according to the present invention, said medicament is used for treating a pathological state caused by abnormal angiogenesis, such as, for example, tumour metastases; arthritic disease; diabetic retinopathy; psoriasis; chronic inflammatory diseases or arteriosclerosis.

In a further embodiment of the present invention, said medicament is used for treating a non-neoplastic disease, such as for example ischemia-induced proliferative retinopathy.

The pharmaceutical compositions will contain at least one compound of Formula (I) as an active ingredient, in an amount such as to produce a significant therapeutic effect. The compositions covered by the present invention are entirely conventional and are obtained with methods which are common practice in the pharmaceutical industry, such as,

for example, those illustrated in *Remington's Pharmaceutical Science Handbook, Mack Pub. N.Y.* – latest edition. According to the administration route chosen, the compositions will be in solid or liquid form, suitable for oral, parenteral or intravenous administration. The compositions according to the present invention contain, along with the active ingredient, at least one pharmaceutically acceptable vehicle or excipient. These may be particularly useful formulation coadjuvants, e.g. solubilising agents, dispersing agents, suspension agents, and emulsifying agents.

The present invention shall now be further illustrated by means of Examples.

General Remarks: ^{1}H- and ^{13}C-NMR spectra were recorded in $CDCl_3$ solution as indicated, at 200 or 300 MHz, respectively. The chemical shift values are given in ppm and the coupling constants in Hz. Optical rotation data were obtained with a Perkin-Elmer model 241 polarimeter. Thin-layer chromatography (TLC) was carried out using Merck precoated silica gel F-254 plates. Flash chromatography was carried out using Macherey-Nagel silica gel 60, 230-400 mesh. Solvents were dried according to standard procedures, and reactions requiring anhydrous conditions were performed under nitrogen. Solutions containing the final products were dried with Na_2SO_4, filtered, and concentrated under reduced pressure using a rotatory evaporator.

Example 1

Synthesis of difluorocombretastatin (Scheme 1)

Synthesis of tert-butyl-dimethyl-sylil isovanillin (1)

To a solution of 6.09 g (40 mmol) of Isovanillin in 50 mL of CH_2Cl_2, were added 6.64 g of TBDMSiCl (44 mmol, 1.1 eq.) and 2.95 g (44 mmol, 1.1 eq) of Imidazole. The solution was stirred at room temperature for three hours and then washed with 0.5 M HCl. The

crude product was purified on a silica gel column using Hexane/Ethyl Acetate 9:1, to give 9 g (33 mmol, 83%) of a colourless oil. Rf = 0.27 (Hex./Ethyl Acetate 95:5)

MS (IS):$[MH]^+$ = 267.2 $[M+Na]^+$ = 289.2 (main peak)

^1H NMR (300 MHz, $CDCl_3$, δ): 0.2 (s, 6H, 2xCH_3), 1.0 (s, 9H, tBu), 3.9 (s, 3H, OCH_3), 6.9-6.95 (d, 1H, CH), 7.4 (s, 1H, CH), 7.45-7.5 (d, 1H, CH), 9.8 (s, 1H, CHO).

^{13}C NMR (75 MHz, $CDCl_3$, δ): -4.4; 18.6; 25.9; 55.6; 111.9; 120.0; 126.5; 130.0; 146.0; 157.1; 191.0.

Synthesis of 2,2-dibromo-2-fluoro-1-(4-methoxy-3-tert-butyl-dimethyl-syliloxyphenyl) ethanol (2)

A mixture of 2.66 g (10 mmol) of TBDMS-Isovanillin and 2.98 g (11 mmol, 1.1 eq) of $CFBr_3$ in 80 mL of Et_2O/THF (1:1) was brought to T = -130°C ; 4.4 mL (11mmol, 1.1 eq) of a 2.5 M BuLi solution in hexane was added to the mixture in 10 minutes. After two hours at T = -70°C, it was necessary to add 1.3 mL of BuLi solution and 0.3 mL of $CFBr_3$ to drive the reaction to completion.

The reaction was quenched with 60 mL of NH_4Cl saturated solution and diluted with 20 mL of diethyl ether. The aqueous phase was back-extracted with 2x20 mL of diethyl ether, the organic fractions were collected and dried over anhydrous sodium sulfate and then purified on a silica gel column using Hexane/Ethyl Acetate 95:5 to give 3.1 g (6.8 mmol, 68 %) of a waxy solid. Rf = 0.5 (Hex./AcOEt 85:15).

MS (IS): [M+Na]+ = 479.1; 481.1; 483.1 (1:2:1)
[M-1]- = 457.2

^1H-NMR (300 MHz, $CDCl_3$, δ): 0.2 (s, 6H, 2xCH_3), 1.0 (s, 9H, tBu), 3.8 (s, 3H, OCH_3), 5.0 (d, 1H, CH, 3JHF = 10Hz), 6.8-6.9 (d, 1H, CHar),

7.0-7.1 (t, 2H, 2xCH).
^{13}C-NMR (75 MHz, $CDCl_3$, δ): 4.4; 18.6; 25.9; 55.6; 82.7; 83.0; 101.3; 105.6; 111.5; 121.3; 122.2; 127.5; 144.9; 152.2.

Synthesis of 1,1-dibromo-1,2-difluoro-2-(4-methoxy-3-tert-butyl-dimethyl-syliloxyphenyl)ethane (3)

(Diethylamino)sulfur trifluoride 1.5 mL (11.2 mmol; 1.8 eq) in 10 mL CH_2Cl_2 was added to a solution of 2.84 g (6.2 mmol) alcohol DA 59 in 14 mL CH_2Cl_2 at -78°C. The reaction mixture was allowed to warm up to 0°C over a period of 2h, quenched with 25 mL of $NaHCO_3$ saturated solution and diluted with 20 mL of diethyl ether. The organic phase was dried over anhydrous sodium sulfate and purified on preparative TLC using Hexane/Ethyl Acetate 98:2 to give 1.8 g (4 mmol; 64.5%) of a yellow oil. Rf = 0.43 (Hex./AcOEt 97:3).

MS (IS): [M+Na]+ = 483.1; 485.1; 487.1 (1:2:1)

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.2 (s, 6H, 2xCH_3), 1.0 (s, 9H, tBu), 3.8 (s, 3H, OCH_3), 5.6 (dd, 1H, CH, 3JHF = 10Hz, 2JHF = 44Hz), 6.8-6.9 (d, 1H, CHar), 7.0-7.1 (t, 2H, 2xCH).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): -4.4; 18.6; 25.9; 55.7; 96; 82.5; 82.8; 95.8; 96.1; 98.3; 98.7; 111.5; 121.1; 121.2; 122.4; 122.5; 124.4; 144.9; 152.8.

Synthesis of 1-bromo-1,2-difluoro-2-(4-methoxy-3-tert-butyl-dimethyl-syliloxyphenyl)ethene (4)

Step 1. Preparation of the Tetramethylpiperidide solution. 1.9 mL (11.7 mmol; 3 eq.) of 2,2,6,6-tetramethylpiperidine was dissolved in 4 mL of anhydrous THF; the solution was cooled to -80°C and then 3.9 mL (9.8 mmol; 2.5 eq) of a 2.5 M solution BuLi in Hexane were added. The mixture was stirred for 2h at 0°C.

Step 2. Dehydrobromination. A solution of 1.8 g (3.9 mmol) of DA 62 in

5mL of anhydrous THF was added to the tetramethyl piperidide solution previously cooled down to -100°C. After 1h the reaction was washed with 10 mL HCl 0.1 N, the aqueous phase was back-extracted with 2x10 mL Et_2O. The organic extracts were collected and dried over anhydrous sodium sulfate and then purified on preparative silica plates with n-Hexane/Ethyl acetate 97:3 to give 857 mg (2.3 mmol; 59%) of product. Rf = 0.8 in Hex./Acetone 8:2.

MS (IS): [M+Na]+ = 401.4; 403.4 (1:1)

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.2 (s, 6H, 2xCH_3), 1.0 (s, 9H, tBu), 3.8 (s, 3H, OCH_3), 6.8-6.9 (d, 1H, CH), 7.1-7.15 (d, 1H, CH), 7.2-7.3 (dd, 1H, CH).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): -4;4; 18.6; 25.9; 55.7; 111.7; 120.5; 121.9; 122.0; 122.1; 124.3; 144.7; 151.9.

Synthesis of (Z)-1,2-difluoro-1-(3,4,5-trimethoxyphenyl)-2-(4-methoxy-3-tert-butyl-dimethyl-syliloxyphenyl)ethene (5)

A mixture of 750 mg (1.98 mmol; 1eq.) of DA 63, 1.260 g (5.94 mmol; 3 eq.) of 3,4,5-trimethoxyphenyl-boronic acid, 4mL of Na_2CO_3 2M aqueous solution and 104 mg (0.09 mmol; 0.05 eq.) of $Pd(Ph_3P)_4$ in 20 mL toluene was refluxed overnight. The solution was then cooled down to room temperature, dried over anhydrous sodium sulfate and the crude mixture was passed through a short silica gel column to remove catalyst. The crude product was purified by chromatography on silica gel plates with Hexane/Acetone 8:2 to give 740 mg (1.6 mmol; 81%) of an oil. Rf = 0.36 in Hex./Acetone 8:2.

MS (IS): $[M+NH_4]^+$ = 484.1; $[2M+NH_4]^+$ = 950.1

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 0.5 (s, 6H, 2xCH_3), 1.0 (s, 9H, tBu), 3.65 (s, 6H, 2xOCH_3), 3.8 (s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 6.5-6.7 (t, 2H, 2xCH), 6.75-7.0 (dq, 3H, 3xCH).

^{13}C-NMR (75 MHz, $CDCl_3$, δ):-4.6;1; 18.6; 25.8; 25.9; 55.7; 56.2; 56.3; 56.4; 61.0; 61.1; 103.4; 105.5; 111.9; 121.0; 122.5; 122.6; 123.8; 145.1; 153.3; 153.8.

Synthesis of (Z)-1,2-difluoro-1-(3,4,5 trimethoxyphenyl)-2-(3-hydroxy-4-methoxyphenyl)ethene (6) ST2303

A 1M solution of Tetrabutylammonium fluoride in THF (9.4 mmol; 2 eq.) was dropped, at 0°C and under inert atmosphere, to a solution of 2.2 g (4.7 mmol) of stilbene DA 64 in 10 mL of anhydrous THF (stored on molecular sieves). The reaction mixture was allowed to warm up to room temperature and after 4h the reaction was complete. The mixture was poured into ice and the aqueous phase extracted with Et_2O (3x20 mL); the organic extracts were collected and dried over anhydrous Na_2SO_4.

The crude mixture was purified by chromatography on silica gel with n-Hexane/Acetone 8:2 to give 1.361 g (3.9 mmol; 83%).

M.p. = 135°C

MS (IS): $[M+H]^+$ = 353.0
$[M+NH_4]^+$ = 370.0
$[M+Na]^+$ = 375.0
$[M-1]^-$ = 351.0

^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.75 (s, 6H, 2xOCH_3), 3.8 (s, 3H, OCH_3), 3.9 (s, 3H, OCH_3), 5.6 (broad, 1H, OH), 6.6 (s, 2H, 2xCH), 6.75-6.8 (d, 1H, CH), 6.85-6.9 (dd, 1H, CH), 7.0 (dd, 1H, CH).

^{13}C NMR (75 MHz, $CDCl_3$, δ): 56.2; 61.1; 105.3; 105.4; 110.4; 114.5; 114.6; 121.1; 123.1; 123.6; 125.1; 125.6; 142.1; 145.6; 147.3; 147.5; 147.6; 153.1.

^{19}F NMR (282 MHz, $CDCl_3$, δ): -126.2 (d, J_{FF} = 14.8Hz), -130.3 (d, J_{FF} = 14.8Hz).

Example 2

Synthesis of difluoronitro- and difluoroaminocombretastatin (Scheme 2)

Synthesis of 2,2-dibromo-2-fluoro-1-(3-nitro-4-methoxy-phenyl)ethanol (7)

A mixture of 978 mg (5.4 mmol) of 3-nitro-4-methoxy-benzaldehyde and 1.6 g (5.9 mmol, 1.1 eq) of $CFBr_3$ in 40 mL of Et_2O/THF (1:1) was brought to T = -130°C ; 3.7 mL (5.9 mmol, 1.1 eq) of a 1.6 M BuLi solution in hexane was added to the mixture in 10 minutes.

The reaction was quenched with 25 mL of NH_4Cl saturated solution and diluted with 20 mL of diethyl ether. The aqueous phase was back-extracted with 2x20 mL of diethyl ether, the organic fractions were collected and dried over anhydrous sodium sulfate and then purified on a silica gel column using Hexane/Ethyl Acetate 95:5 to give 1.146 g (3.1 mmol, 57.4 %) of a yellow oil. R_f = 0.53 (Hex./AcOEt 6:4).

MS (IS): $[M-1]^-$ = 371.8
$[M+AcO]^-$ = 431.7

^1H-NMR (300 MHz, $CDCl_3$, δ): 3.2 (bs, 1H, OH), 4.0 (s, 3H, OCH_3), 5.1-5.2 (m, 1H, CH), 7.05-7.15 (d, 1H, CH_{ar}) 7.7-7.8 (d, 1H, CH_{ar}), 8.05 (s, 1H, CH_{ar}).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 56.9; 81.5; 81.8; 98.9; 100.3; 104.6; 113.3; 126.3; 127.3; 134.3; 153.8.

Synthesis of 1,1-dibromo-1,2-difluoro-2-(3-nitro-4-methoxy-phenyl) e-thane (8)

(Diethylamino)sulfur trifluoride 730 µL (5.58 mmol; 1.8 eq) in 5 mL CH_2Cl_2 was added to a solution of 1.146 g (3.1 mmol) of the alcohol (7) in 7 mL CH_2Cl_2 at –78°C. The reaction mixture was allowed to warm up to 0°C over a period of 2h, quenched with 15 mL of $NaHCO_3$ saturated solution and diluted with 20 mL of diethyl ether. The organic phase was dried over anhydrous sodium sulfate and purified by chromatography on SiO_2 using Hexane/Ethyl Acetate 7:3 to give 960 mg (2.6 mmol; 84 %) of a yellow oil. R_f = 0.493 (Hex./AcOEt 7:3)

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 4.0 (s, 3H, OCH_3), 5.55-5.80 (dd, 1H, CH,), 7.1-7.2 (d, 1H, CH_{ar}), 7.7-7.8 (d, 1H, CH_{ar}), 8.1 (s, 1H, CH_{ar}).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 29.9; 56.9; 94.4; 94.8; 97.0; 97.2; 97.4; 113.6; 124.1; 124.4; 126.2, 134.0, 139.4; 154.5.

Synthesis of (E)-1-bromo-1,2-difluoro-2-(3-nitro-4-methoxy-phenyl)-ethene (9)

Step 1 Preparation of the Tetramethyl-piperidide solution. 1.3 mL (7.8 mmol; 3 eq.) of 2,2,6,6-tetramethyl-piperidine was dissolved in 3 mL of anhydrous THF.; the solution was cooled to -80°C and then 3.9 mL (9.8 mmol; 2.5 eq) of a 2.5 M solution BuLi in Hexane were added. The mixture was stirred for 2h at 0°C.

Step 2 Dehydrobromination. A solution of 960 mg (2.6 mmol) of (8) in 5mL of anhydrous THF was added to the tetramethyl piperidide solution previously cooled down to -100°C. After 1h the reaction was washed with 10 mL HCl 0.1 N, the aqueous phase was back-extracted with 2x10 mL Et_2O. The organic extracts were collected and dried over anhydrous sodium sulfate and then purified on silica gel with n-Hexane/Ethyl acetate 8:2 to give 100 mg (0.34 mmol; 13%) of product. R_f = 0.36 in Hex./Acetone 8:2.

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 4.0 (s, 3H, OCH_3), 7.1-7.2 (d, 1H, CH_{ar}), 7.8-7.9 (d, 1H, CH_{ar}), 8.2 (s, 1H, CH_{ar}).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 57.0; 113.6; 113.8; 120.5; 120.9; 124.6; 125.1; 125.4; 126.2; 126.3; 128.8; 129.3; 133.3; 134.0; 141.1; 141.3; 144.4; 144.6; 154.0.

Synthesis of (Z)-1,2-difluoro-1-(3,4,5-trimethoxyphenyl)-2-(3-nitro-4-methoxy-phenyl)ethene (10)

A mixture of 90 mg (0.31 mmol; 1eq.) of (9), 198 mg (0.93 mmol; 3 eq.) of 3,4,5-trimethoxyphenyl-boronic acid, 0.6 mL of Na_2CO_3 2M aqueous solution and 19 mg (0.0016 mmol; 0.05 eq.) of $Pd(Ph_3P)_4$ in 4 mL toluene was refluxed for 2.5 h. The solution was then cooled down to room temperature, dried over anhydrous sodium sulfate and the crude mixture was passed through a short silica gel column to remove catalyst. The crude product was purified by chromatography on silica gel with Hexane/Acetone 8:2 to give 57 mg (0.15 mmol; 48 %) of a yellow oil. R_f = 0.17 in Hex./Acetone 8:2.

MS (IS): $[M+H]^+$ = 382.4; $[M+NH_4]^+$ = 399.3.

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 3.75 (s, 6H, 2xOCH_3), 3.85 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 6.6 (s, 2H, 2xCH_{ar}), 6.95-7.05 (dq, 1H, CH_{ar}), 7.4-7.5 (d, 1H, CH_{ar}), 7.9 (s, 1H, CH_{ar}).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 29.9; 56.4; 56.9; 61.2; 105.9; 113.6; 125.1; 133.3; 153.7.

Synthesis of (Z)-1,2-difluoro-1-(3,4,5-trimethoxyphenyl)-2-(3-amino-4-methoxyphenyl)ethene (ST2578)

To a solution of 40 mg (0.105 mmol) of nitro-stilbene (10) in AcOH (5 mL) was added zinc powder 75 mg (1.15 mmol; 11 eq.); the mixture was stirred at room temperature for 1.5 h. The reaction mixture was filtered over Celite and the filtrate evaporated to dryness.

The crude product was purified by chromatography on silica gel with CH_2Cl_2, then on preparative HPLC to give 32 mg (0.091 mmol; 87 %) of a white solid. R_f= 0.31 in CH_2Cl_2.

A small portion (4 mg) of the trifluoroacetate salt obtained from the prep. HPLC was passed through an ion-exchange column IRA402*Cl^- to give 3 mg of the corresponding HCl salt (ST2578).

MS (IS): $[M+H]^+$ = 352.3; $[2M+H]^+$ = 703.1.

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 3.7 (s, 6H, 2xOCH_3), 3.85 (s, 3H, OCH_3), 4.0 (s, 3H, OCH_3), 6.6 (s, 2H, 2xCH_{ar}), 6.7-6.8 (t, 1H, CH_{ar}), 6.85-6.90(d, 1H, CH_{ar}), 6.95 (s, 1H, CH_{ar}).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 29.9; 55.9; 56.3; 56.5; 61.1; 105.0; 105.4; 110.5; 116.6; 121.6; 122.8; 123.1; 125.5; 125.9; 133.0; 143.1; 143.9; 146.6; 149.5; 153.3; 153.7.

Example 3

Synthesis of monofluorocombretastatins (Scheme 3)

Convenient approaches to the synthesis of both of the regioisomeric monofluorocombretastatins, starting from the natural CA-4, are the following:

A) Bromofluorination of the CA-4, followed by base-promoted HBr elimination (*Giannini, G., Gazz. Chim. It., 1997, 127, 545; Thakker D.R., et al., J. Org. Chem., 1989, 54, 3091*).

B) Fluorination, by DAST, of the bromohydrin obtained from the CA-4, followed by base-promoted HBr elimination.

C) Synthesis of the epoxide from the CA-4, epoxide opening to obtain:

- the bromohydrin and to continue as point B), or

the fluorohydrin followed by elimination of opportune hydroxyl derivative.

Example 4

Synthesis of disodium-phosphate prodrug of difluorocombretastatin (Scheme 4)

A typical procedure for the synthesis of the disodium-phosphate prodrug is well known in the literature (*Pettit, G.R., et al., Anti-Cancer Drug Design 1998, 13, 183-191*) and is intended to be generally applicable to all the compounds here described, possessing a free phenolic moiety. As an example, the synthesis of the disodium-phosphate prodrug of compound (6) is here reported.

Synthesis of (Z)-1,2-difluoro-1-(3,4,5-trimethoxyphenyl)-2-(3-hydroxy-4-methoxyphenyl)ethene o-dibenzyl-phosphate (11)

To a solution of 30 mg of (6) (0.09 mmol) in 1 mL dry CH_3CN, cooled down to -25°C, 44 μL (0.45 mmol; 5 eq.) CCl_4 were added. After 5 minutes mixing 33 μL (0.19 mmol; 2.1 eq.) diisopropyl-ethyl amine, 1 mg (0.009; 0.1 eq.) DMAP and 29 μL of di-benzyl phosphite were added to the solution and the reaction mixture was stirred for 1.5 h at -10°C. The reaction was quenched by pouring 5 mL KH_2PO_4 0.5 M; the aqueous phase was washed with AcOEt (3x10mL) and the organic phase was back-extracted with 10 mL H_2O and then with 10 mL NaCl saturated solution. The crude mixture was purified by silica gel chromatography with Hexane/AcOEt 6:4 to give 55 mg of a colourless oil (0.088; 98%). R_f = 0.32 in Hex./AcOEt 6:4

MS (IS): $[M+H]^+$ = 613.4; $[M+NH_4]^+$ = 630.2; $[M+Na]^+$ = 635.0.

^{1}H-NMR (300 MHz, $CDCl_3$, δ): 3.70 (s, 6H, 2xOCH_3); 3.80 (s, 3H, OCH_3); 3.85 (s, 3H, OCH_3); 5.65 (s, 2H, CH_2); 5.70 (s, 2H, CH_2); 6.55 (s,

2H, 2xCHar.); 6.80-6.85 (d, 1H, CHar.); 7.10-7.15 (dd, 1H, CHar.); 7.25 (s, 1H, CHar.); 7.35-7.45 (m, 10H, CHar.).

^{13}C-NMR (75 MHz, $CDCl_3$, δ): 29.6; 29.9; 56.1; 56.3; 56.4; 61.1; 70.1; 70.3; 104.3; 105.4; 106.4; 109.1; 112.6; 115.1; 121.7; 125.0; 126.6; 128.1; 128.2; 128.8; 128.9; 129.2; 130.9; 132.3; 135.7; 145.5; 153.2; 153.4.

Synthesis of (Z)-1,2-difluoro-1-(3,4,5-trimethoxyphenyl)-2-(3-hydroxy-4-methoxyphenyl)ethene o-disodium phosphate (12) ST2493

To a solution of 50 mg (0.08 mmol) of (11) in 1.5 mL dry CH_3CN in a three-necked round-bottom flask and under an Ar atmosphere, 24 mg (0.16 mmol; 2 eq.) of NaI were added. The mixture was stirred at room temperature for 10 minutes and then a solution of 20 µL $(CH_3)_3SiCl$ (0.16 mmol; 2eq.) in 1 mL CH_3CN was dropped in.

After 1.5 h one equivalent of NaI and one equivalent of $(CH_3)_3SiCl$ were added to complete the reaction. Water (just enough to dissolve the salts) was added and the pale yellow colour removed by the addition of 10% aq. $Na_2S_2O_3$ (1 mL). The organic phase was separated and the aqueous phase extracted with AcOEt (4x4mL). The combined organic extracts were concentrated to give a yellow waxy solid.

The solid was dissolved in 1.5 mL dry MeOH (stored on molecular sieves) and 9 mg (0.16 mmol; 2 eq.) of sodium methoxide were added and the solution stirred at room temperature for 12 h. The methanol was removed in vacuo and the solid recrystallized from water-acetone and methanol-acetone to give 35 mg (0.073 mmol; 91%) of compound (12) as a white solid.

^{1}H-NMR (200 MHz, D_2O, δ): 3.60 (s, 6H, 2xOCH_3); 2.70 (s, 3H, OCH_3); 3.8 (s, 3H, OCH_3); 6.70 (bs, 2H, 2xCHar.); 6.85 (bs, 2H, 2xCHar.); 7.55 (s, 1H, CHar.).

Example 5

Synthesis of bromofluorocombretastatin (Scheme 5)

Synthesis of bromofluorocombretastatin

1. Acetylation: To a solution of 32 mg (0.1 mmol) of combretastatin in 2 ml of dry pyridine, 20 μl of Ac_2O were added at r.t.

After 1.5 h the reaction was stopped by diluting with 20 ml of AcOEt, washed with 3N HCl solution and dried over Na_2SO_4.

The crude product was purified by chromatography (eluted with Hexane:AcOEt=8:2) to give 30 mg of acetylated combretastatin.

2. Bromofluorination: To a solution of 19 mg (0.11 mmol) of NBS and 105 ml of HF.Pyr in Et_2O/CH_2Cl_2 was added a solution of 30 mg (0.084 mmol) of acetylated combretastatin in 2.5 ml of dry Et_2O at -50°C.

After 1 h the temperature of reaction mixture was raised at r.t. and left for 3.5h.

The reaction mixture was poured into water and extracted with Et_2O and dried over Na_2SO_4.

The crude product was purified by chromatography (eluted with Hexane:AcOEt = 7:3) to give 16 mg of bromofluoro acetylated combretastatin.

3. Deprotection: The acetylated derivative was dissolved in 2 ml of MeOH and added with 2N HCl (1ml). The reaction was completed in 2 h at r.t. The product was isolated with 92% yield.

Cell culture and cytotoxicity assay

Primary cultures of bovine microvascular endothelial cells (BMEC) were obtained from bovine adrenal glands as described by Folkman (*Folkman J., Haudenschild C.C., Zetter B.R. Long-term culture of capillary endothelial cells. Proc. Natl. Acad. Sci. U S A, 1979 Oct; 76(10): 5217-21*). BMEC were maintained in DMEM supplemented with 20% fetal calf serum (FCS), 50 units/ml heparin (Sigma, St. Louis, MO), 50 μg/ml bovine brain extract, 100 units/ml gentamycin. HUVEC (Human umbilical vein endothelial cells) were obtained from BioWhittaker (Walkersville, MD) and grown in EGM-2 (BioWhittaker). EA-hy 926 cell line, a HUVEC-adenocarcinoma immortalized cell hybrid, was obtained from the Dipartimento di Scienze Biomediche e Oncologia Umana (Università di Bari, Italy), and cultured in DMEM supplemented with 10% serum and 50 μg/ml gentamycin sulfate.

The following cell lines were purchased from ATCC and cultured according to manufacturer's instructions: NCIH460 human lung carcinoma and MeWo human melanoma. HT-29 colon adenocarcinoma cells, obtained from Istituto Nazionale Tumori (Milan, Italy), were grown in RPMI 1640 (GIBCO) containing 10% fetal bovine serum (GIBCO) and 50 μg/ml gentamycin sulfate.

To test the effects of ST2303 on growth, cells were seeded in 96-well tissue culture plates (Corning) at approximately 10% confluence and were allowed to attach and recover for at least 24 h. Varying concentrations of the compound were then added to each well. The plates were incubated for 24 h and then washed before incubating them for additional 48 h. The number of surviving cells was then determined by staining with sulforhodamine B as described by Skehan et al. (1990). ST2303 inhibitory concentration 50 (IC_{50}) ± SD on different cell lines, evaluated by "ALLFIT" computer program, are shown in Table 1.

Table 1

Cell line	IC_{50} ± SE (nM)
BMEC	5±0.5
HUVEC	1±0.3
EAHY.926	5±0.5
NCI-H460	3±0.005
HT29	>200
MeWo	4±0.0003

Tumor growth evaluation

NCI-H460 human lung carcinoma from in vitro cell cultures were injected s.c. ($3x10^6$ cells/100 µl/mouse) into the right flank of CD-1 nude mice. Four days after tumor implant mice started to be treated with ST2493 at the dose of 50 mg/kg according to the following schedule: qdx5/w. CA-4P (combretastatin A-4 P) at the same dose was used as positive control.
All animals were weighed during the whole treatment period, in order to adjust the volume of drug administration and to record the percent of body weight loss due in the course of treatment.

Tumor growth was assessed by twice a week measurements of the shortest (width) and the longest (length) diameters of each tumor by a Vernier caliper and the antitumor activity was evaluated in terms of percent inhibition of tumor growth. Tumor volume (or tumor weight*) was calculated according to the following formula using caliper measurements: tumor volume or TV (mm^3) = [length (mm) x width $(mm)^2$]/2.

Tumor volume inhibition percent (% TVI) was calculated according to the equation: 100 – [(mean tumor volume of treated group/mean tumor volume of control group) x 100]. A P value ≤ 0.05 was considered statistically significant.

Results, reported in Table 2, show that intraperitoneal administration of ST2493 determined a significant TVI compared to vehicle. The TVI obtained with ST2493 was significantly greater (P=0.02) than that obtained with CA-4P.

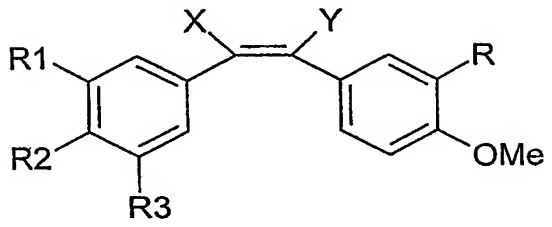
Table 2

Treatment	n	% BWL	mortality	% TVI Days after cell injection		
Vehicle	8	0	0/8	/	/	/
ST2493 i.p. 50mg/kg	8	8	0/8	0	54*	68* ^
CA-4P i.p. 50mg/kg	8	3	0/8	0	11	47*

*P<0.01 vs vehicle; ^P=0.02 vs ST2494

CLAIMS

1. A compound of formula (I)



wherein:

R_1, R_2 and R_3, which can be the same or different, are H, OMe, NO_2, NHR';

X and Y are halogen or H, but cannot be both H;

R = OH, OPO_3Na_2, NO_2, NHR';

R' = H, alkyl (C_1-C_6), $(COCHR''NH)_n$-H;

R'' = H, an amino acid side chain, Ph;

n an integer comprised between 1 and 3;

their pharmaceutically acceptable salts, racemates and single enantiomers.

2. A compound according to Claim 1, selected from the group consisting of:
a compound wherein at least one of X and Y is halogen, R_1-R_3 are methoxy, and R is hydroxy;
a compound wherein at least one of X and Y is halogen, R_1-R_3 are methoxy, R is amino or substituted amino;
a compound wherein at least one of X and Y is halogen, R_1-R_3 are different from methoxy, R is hydroxy;

a compound wherein R is OPO_3Na_2 and
a compound wherein R' is $(COCHR"NH)_n$-H.

3. A compound according to Claim 1 or 2, wherein R" is the side chain of a natural amino acid.

4. A compound according to Claim 1 selected from the group consisting of:

difluorocombretastatin;
difluoroaminocombretastatin;
monofluorocombretastatin;
monofluorocombretastatin;
bromofluorocombretastatin;
monofluoroaminocombretastatin;
monofluoroaminocombretastatin.

5. A process for the preparation of the compounds of Claim 1, wherein X and Y are both F comprising the following steps:
a) reaction of 1-bromo-1,2-difluoro-2-(4-methoxy-3-(protected OH)-phenyl)ethene with 3-R_1-4-R_2-5-R_3-phenylboronic acid, and
b) restoring the 3-(protected OH) group.

6. A process for the preparation of compounds of Claim 1, wherein one of the X and Y is F and the other one is hydrogen, comprises the following steps:
a) bromofluorination of the compound of Formula (I), wherein X and Y are H, and
b) base-promoted HBr elimination.

7. A process for the preparation of compounds of Claim 1, wherein one of the X and Y is F, comprising the following steps:
a) transformation of compound of Formula (I), wherein X and Y are H into the respective bromohydrin, and
b) base-promoted HBr elimination.

8. A process for the preparation of compounds of Claim 1, wherein one of the X and Y is F, comprising the following steps:

a) transformation of compound of Formula (I), wherein X and Y are H into the respective epoxide;

b) epoxide opening to give the respective bromohydrin, and

c) base-promoted HBr elimination, or in alternative,

d) epoxide opening to give the respective fluorohydrin, and

e) elimination of the opportune hydroxyl derivative.

9. A process for the preparation of compounds of Claim 1, wherein one of the X or Y is F and the other is Br, comprising the following steps:

a) transformation of compound of Formula (I), wherein X and Y are H into the respective bromohydrin, and

b) base-promoted HBr elimination.

10. The use of the compounds of any one of Claims 1-4 for the recognition and binding to the tubulin site.

11. The use of the compounds of any one of Claims 1-4 as medicaments.

12. The use of the compounds of any one of Claims 1-4 for the preparation of a medicament for treating pathological states which arise from or are exacerbated by cell proliferation.

13. The use according to Claim 12, wherein said pathological state is a tumour.

14. The use according to Claim 13, wherein said tumour is selected from the group consisting of sarcoma, carcinoma, carcinoid, bone tumour, neuroendocrine tumour, lymphoid leukaemia, acute promyelocytic leukaemia, myeloid leukaemia, monocytic leukaemia, megakaryoblastic leukaemia and Hodgkin's disease.

15. The use according to Claim 12, wherein said pathological state is caused by abnormal angiogenesis.

16. The use according to Claim 15, wherein said pathological state caused by abnormal angiogenesis is selected from the group consisting of tumour metastases; arthritic disease; diabetic retinopathy; psoriasis; chronic inflammatory diseases or arteriosclerosis.

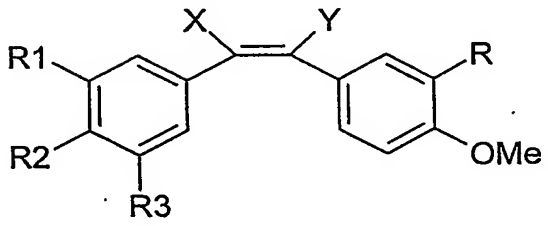
17. The use according to Claim 12, wherein said pathological states is a non-neoplastic disease.

18. The use according to Claim 17, wherein said disease is ischemia-induced proliferative retinopathy.

19. A pharmaceutical composition comprising at least a compound of any one of Claims 1-4, in admixture with at least one pharmaceutically acceptable carrier and/or excipient.

Abstract

The present invention is related to new derivatives of Combretastatin, of Formula (I)



obtained by total synthesis. The strategy developed for each of the compounds is to i) replace a halogen (i.e. fluorine atom) to hydrogen on olefinic bound; ii) replace an aromatic ring in a natural product with an amino-aromatic ring. Said compounds recognize and bind the tubulin site: are useful for treating pathological states which arise from or are exacerbated by cell proliferation - as anticancer and/or antiangiogenic activity, in a mammal - to pharmaceutical compositions comprising these compounds.

FIGURE 1

Scheme 1: synthesis of difluorocombretastatin

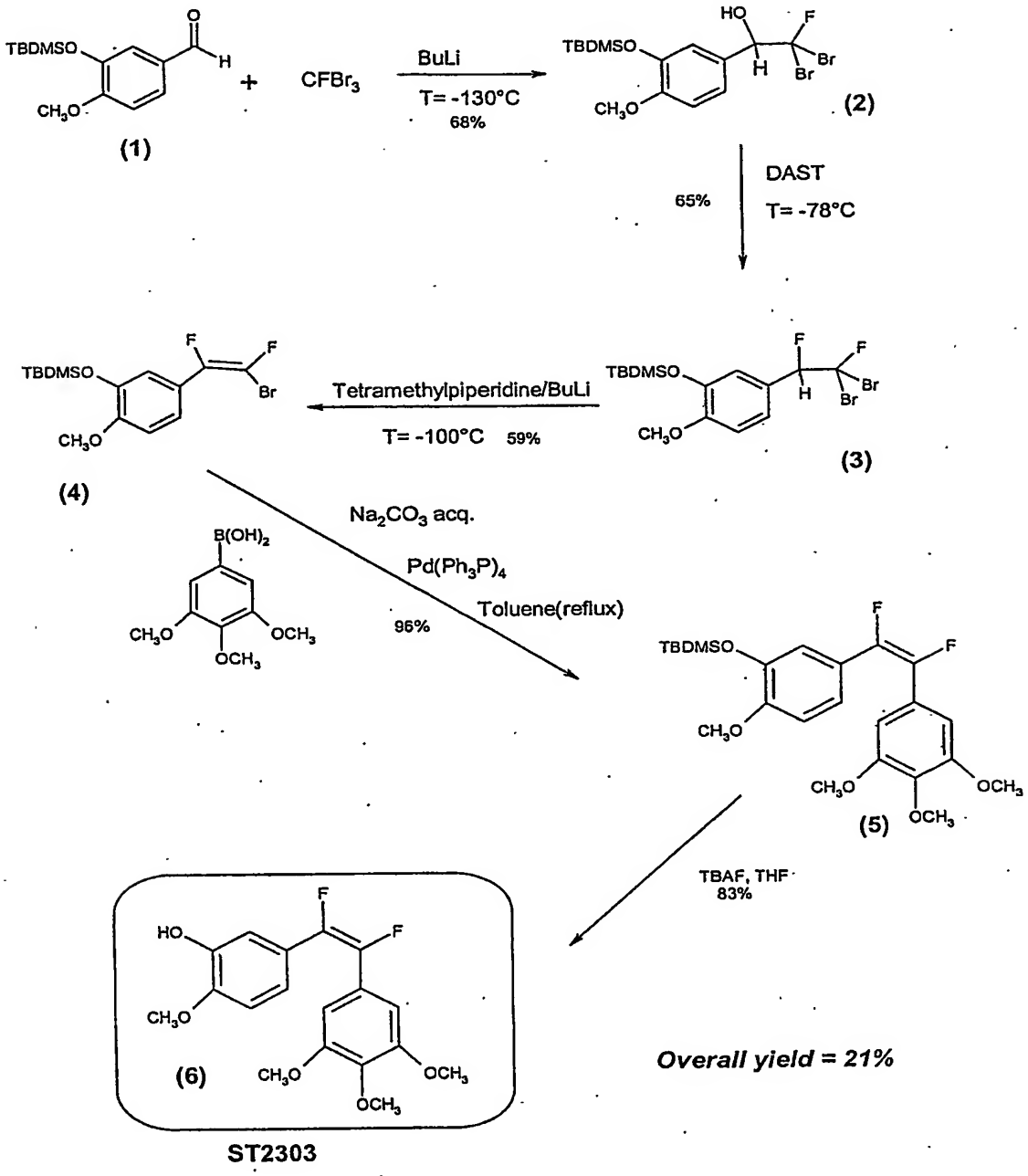


FIGURE 2

Scheme 2: Synthesis of difluoro-Nitro- and difluoro-Amino-combretastatin

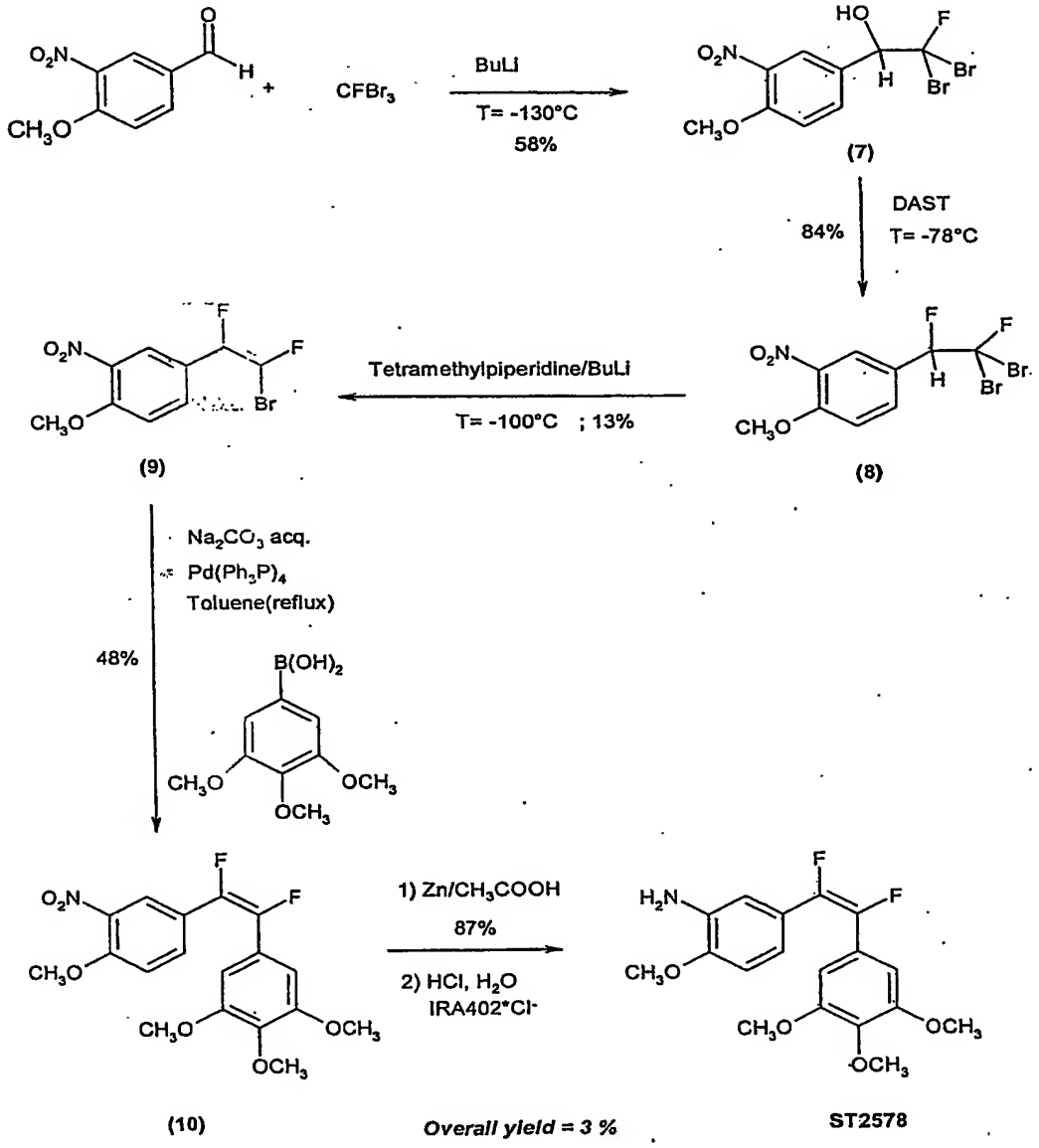


FIGURE 3

Scheme 3: Synthesis of monofluorocombretastatins

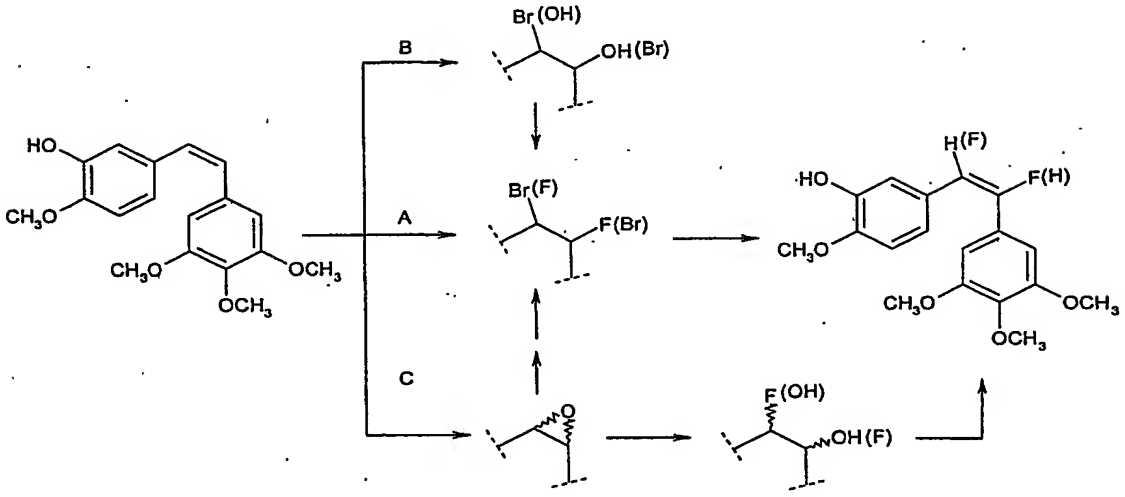


FIGURE 4

Scheme 4: Synthesis of disodium-phosphate prodrug difluoro-combretastatin (ST2493)

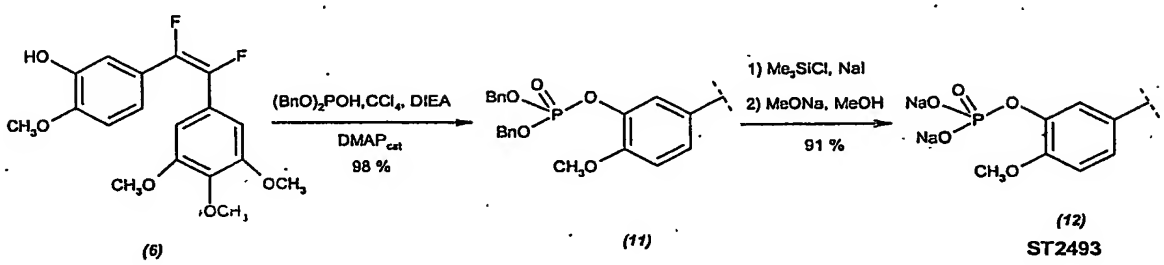
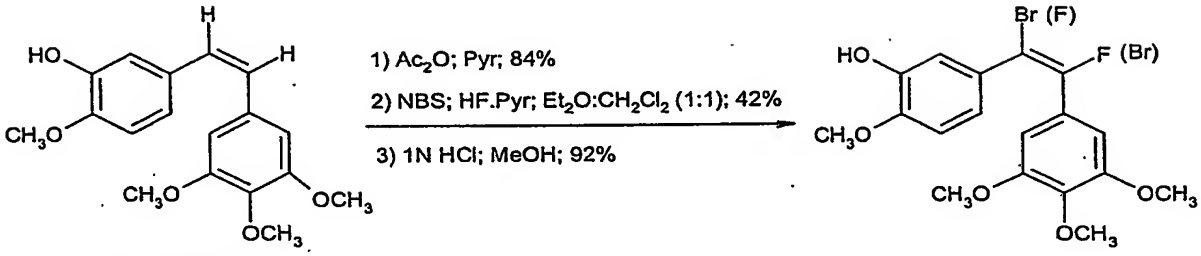


FIGURE 5

Scheme 5: Synthesis of bromofluorocombretastatin



(Combretastatin)